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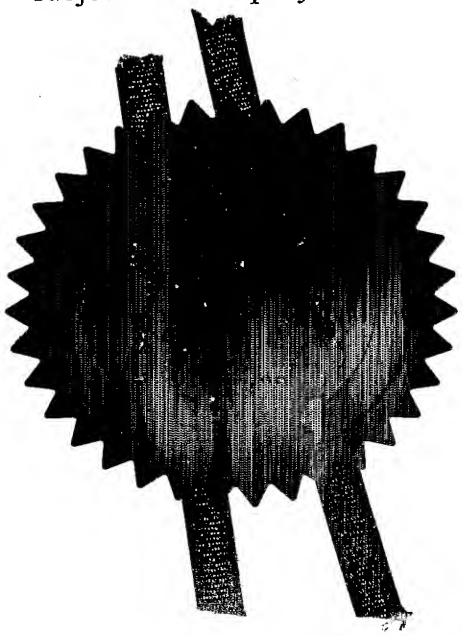

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PAUL DOUGLAS CLARKE
201 EAST DULWICH GROVE
LONDON
SE22 8SY

Patents ADP number *(if you know it)* 7319379001

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4. Title of the invention — ANTIVIRAL COMPOSITION

5. Name of your agent *(if you have one)* — A A THORNTON & CO

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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ANTIVIRAL COMPOSITION

The present invention relates to an antiviral composition.

It is known that a number of natural products have insect repellent properties. Citronella oil which is obtained from certain grasses is one example of such a natural product and oil from the Neem tree is another. We have previously investigated Eucalyptus citriadora and found that it possesses insect repellent properties. The repellent properties are found in a fraction rich in p-menthane-3,8-diol (PMD). This is described in our GB-A-2282534. In GB-A-1315625, there is described the use of certain p-menthane diols, but not PMD (i.e. p-menthane-3,8-diol), to provide a physiological cooling effect. EP-B-1204319 describes the use of PMD as a general antiseptic. We have now found, very surprisingly, that PMD also possesses the totally unrelated quality of antiviral properties.

According to one aspect of the invention, we provide the use of PMD in the manufacture of a medicament for use as an antiviral agent.

According to another aspect of the invention, we provide the use of PMD to destroy or inactivate viruses.

According to a further aspect of the invention, we provide the use of PMD in the manufacture of a medicament for the treatment of diseases caused by viruses having a lipid envelope.

It is to be understood that the term "virucidal" means having the capacity to destroy or inactivate viruses. It is also to be understood that the term "antiviral" means having the capacity to inhibit or stop the growth and reproduction of viruses, or having the capacity to destroy or inactivate viruses. The use of PMD in the present invention may be virucidal or antiviral.

The PMD for use in the present invention may be derived from a natural source or may be synthetic, or a mixture of the two. A preferred source of natural PMD is the lemon eucalyptus plant Eucalyptus citriadora. Synthetic PMD may be obtained by any route, for example, such as described by Zimmerman and English in J.A.C.S. 75 (1953) pp 2367-2370. PMD is also a precursor obtained during the synthesis of menthol. The precursor is usually in the form of a specific isomer of PMD.

The PMD for use in the present invention may be a substantially pure form of the compound, or a crude extract, for example from a natural source. An example of a

crude extract is a PMD-rich extract derived from lemon eucalyptus by acid modification of lemon eucalyptus oil. The PMD can be produced by cyclisation of citronellal which is present in high concentration in lemon eucalyptus oil (approximately 75% by weight). We have obtained a PMD-rich extract from the lemon eucalyptus oil which includes both geometric isomers of PMD, usually at about 64% by weight. The crude extract also includes citronellol and isopulegols plus certain other minor components.

According to a further aspect of the invention, there is provided the use of a composition comprising a PMD-rich extract, which is derived from natural lemon eucalyptus oil, as an antiviral agent. An example of this sort of crude extract is available under the trade mark "Citriodiol".

A composition for use in accordance with the invention can comprise PMD and a carrier. PMD is poorly soluble in water, so it is preferred to use an oil as a carrier, or to use a solvent, such as alcohol, for water-based compositions.

It is known that PMD exists in two geometric isomeric forms, namely the cis and trans isomers. Altogether, there are 8 isomers of PMD, as shown in Figure 1. This invention encompasses any single one isomer and also any combination of one or more isomers.

Our experimental work is based on the 98% pure cis isomer. It will be understood, however, that the claimed activities for PMD are common to all its isomeric forms. Thus, the PMD may be used in the form of a single pure cis or trans isomer, or in the form of a mixture of the isomers, with any appropriate proportion of each isomer. For example, a 50:50 mixture could be used.

In a further aspect of the invention, the composition for use in the invention comprises only one of the isomers of PMD, with a carrier therefor.

It is a further aspect of the invention that the relative amounts of cis: trans PMD isomers in the compositions for use in the present invention are varied as desired. This can be done by mixing previously separated isomers in the appropriate ratio, or by adjusting the ratio in a mixture of naturally occurring PMD or PMD from a synthetic source.

In tests we have found that PMD is effective against influenza, as exemplified by the effectiveness of PMD against the influenza virus A/Sydney/5/97. We have also found that PMD is effective against Urbani Severe Acute Respiratory Syndrome (Urbani SARS) and Herpes caused by the Herpes Simplex Virus type-1 (HSV-1).

In an embodiment of the present invention, therefore, the PMD is used to treat influenza. In a further embodiment, the PMD is used to treat influenza caused by the virus A/Sydney/5/97. In another embodiment, the PMD is used to treat Urbani SARS. In another embodiment, the PMD is used to treat Herpes caused by Herpes Simplex Virus type-1 (HSV-1).

Influenza viruses, including A/Sydney/5/97 and viruses which fall within the A and B types of influenza, Urbani SARS and Herpes Simplex virus type-1 all possess a lipid envelope. Other examples of viruses having a lipid envelope include coronaviruses (one of which is Urbani SARS), Herpes Simplex Virus type-2 (HSV-2), Human Immunodeficiency Virus (HIV), Hepatitis B, Hepatitis C, West Nile virus, Vesicular stomatitis virus, Sindbis virus and Sendai virus.

The uses of the present invention may be adopted in sanitizing a surface, for example in a hospital room or ward. In such cases PMD is applied to the surface. The PMD is preferably either in solution or present as an emulsion in suitable liquid carriers. Most desirably, the PMD is formulated for spray application. For example, the PMD or Citriodiol can be dissolved in a suitable solvent or solvent mixture. For example, in one embodiment, the PMD may be provided in the form of a nasal spray; in another embodiment, the PMD may be provided in the form of a spray for telephones.

In one mode of application, the spray is an electrostatic spray. For electrostatic spraying, the solvent or solvent system will need to be appropriate for electrostatic spraying, as will be clear to those skilled in the art. It is preferable to use a mixture of conductive and nonconductive solvents to achieve a sprayable solution with the appropriate electrical resistivity for the spray nozzle in question, but suitable single solvents can be used. Charged particles of the composition including PMD are projected as a fine mist and because all the particles carry a similar, for example positive, charge, they repel each other, but are attracted to an oppositely charged surface. By this means of spraying, a very good coverage of the composition on the surface may be obtained. Devices for electrostatically spraying the composition for use in the invention will be known to the person skilled in the art.

To increase the likelihood of the charged particles covering the skin surface, the electrostatic spray nozzles may desirably be arranged to spray into the interior of a cabinet or container as the hand is introduced therein.

An electrostatic spray or a simple atomised spray may also be used, for example, for dispensing a composition including PMD onto a hand (or other part) of a person. The actuation of the dispenser may be by means of an infra-red sensor, for example, so that the person need not contact a surface, and thereby risk the transfer of viruses to or from their hand. Spray application to a hand may be used, with advantage, where a substantially uniform coverage of antiviral agent is particularly important e. g. to a surgeon during "scrubbing up" before surgery.

The liquids for applying to a surface, by spraying or otherwise, in accordance with the invention may contain, apart from the solvent(s) and/or other liquid carrier(s), other components as necessary or desirable for the intended purpose. Thus, second or further antiviral agents may be included, as may surfactants, fragrances etc. In general, the compositions may be identical to known compositions for the purpose except that they contain PMD in addition to, or in whole or part substitution for one or more of, the other ingredients.

The amount of PMD required to have an antiviral effect may vary widely between different viruses and also on the time in contact with the virus. Thus, we have shown that at 10 seconds' contact time, at least a 1% w/v PMD concentration is desirable to have a significant antiviral action against A/Sydney/5/97 Influenza virus, while for Urbani SARS even 0.25% for 10 seconds' exposure is effective. In contrast, at least 1% w/v PMD is desirable for the prolonged contact time of 5 minutes to have a significant effect on the Herpes virus HSV-1 in our laboratory tests. Thus routine experimentation is required to decide the optimum concentration of PMD and the optimum time for contact for any specific virus.

PMD may also be included as an antiviral agent in household detergents, cleansers and creams, for example, washing powders or conditioners and hand gels. Again, the PMD may be included in what are otherwise standard or known compositions for the purpose concerned. The PMD may be an extra ingredient or in partial or complete replacement of a standard ingredient. The compositions may already contain an antiviral agent and the PMD is added to give an extra antiviral effect.

Furthermore, PMD may be impregnated into household objects which may be prone to virus infestation and so risk infecting inhabitants, e. g. dishcloths, plastic soap dishes, surfaces used for the preparation of food.

For these purposes, the PMD may be included during manufacture of the object, e. g. in mixtures for plastics mouldings or the like, or it may be applied to the object after manufacture, e. g. by soaking dishcloths in PMD. The presence of the PMD at the surface of the object will provide the desired antiviral effect. This is particularly useful for work surfaces, although such surfaces can also be regularly treated with PMD, by spraying or otherwise.

PMD may be sprayed onto face mask material or impregnated into such material during manufacturing to prevent both ingress and egress of viable viral particles, towards or away from the individual. Thus the individual may be protected from viruses transmitted from another infected person or may use the mask to prevent his own infection being passed on to others. This would be of particular use against those viruses spread by droplet spray or aerosol. The PMD spray may be applied to either the outside or inside of the mask, or both. In masks made with replaceable filters, the filters may be similarly sprayed or impregnated during manufacture to allow replenishment in the most cost effective way. This would particularly apply to masks now being designed to look attractive and be reused repeatedly with replaceable filters. It may also be possible to impregnate the filters within air conditioning systems whether in hotels or in aeroplanes or other vehicles where public use is heavy.

Additionally, PMD may be incorporated into prewetted wipes, for use, for example, in cleaning masks, lavatory seats, door handles or elevator buttons.

A composition including PMD can also be used in medicine. For example, it can be applied to broken skin, or to internal mucous membranes. It may be an ingredient in throat lozenges or pastilles or other products for ingestion. In medical uses the PMD may be formulated with the carrier as a cream, or, as mentioned above, as a throat lozenge or pastille. A composition including PMD may be applied to the accessible inner surfaces of the nose in order to control or eliminate viruses which may cause regular systemic effects. For these purposes, PMD may be formulated as a nasal spray. Another specific medical use is in wound irrigation during surgery, e. g. surgery conducted on the peritoneal cavity.

As will be evident to those skilled in the art, there are a very large number of medical uses of PMD as an antiviral or virucidal agent. In general, new formulations for these purposes are not required: it is adequate and satisfactory to take a known or standard composition and include the PMD therein. Alternatively, one or more

ingredients may be replaced by the PMD as appropriate. Those skilled in the art will well know the make-up of the various compositions and no further particular description thereof is given here.

PMD is the active ingredient in the insect repellent sold under the trade name "Mosiguard"™. Extensive tests have already been conducted to show regulatory authorities that PMD is not toxic. Mosiguard insect repellent has been marketed for several years and there has been no report of any significant toxicity thereof. Potentially, therefore, the medical uses of PMD may be topical or systemic. Systemic administration may be by way of an oral dosage form or by a parenteral route, such as by intra-venous injection.

In general, PMD is used in accordance with the invention in a wide variety of vehicles, depending on the particular use intended. The vehicles may, for example, include solids, liquids, emulsions, foams and gels.

Typical vehicles include aqueous or alcoholic solutions, oils, fats, fatty acid esters, long chain alcohols and silicone oils, finely divided solids such as starch or talc, cellulosic materials and aerosol propellants. Topical compositions include perfumes, powders and other toiletries, lotions, liniments, oils and ointments, for example. Toiletries generally include after shave lotions, shaving soaps, lipstick, creams, foams, toilet water, deodorants, antiperspirants, solid colognes, toilet soaps, bath oils and salts, shampoos, face and hand creams, cleansing tissues, mouthwashes, eye drops, for example. Medicaments and allied compositions include, for example, ointments, lotions, decongestants and throat lozenges. The amount of PMD present in the compositions will be selected to give the desired effect but we believe that generally up to 5.0 wt%, preferably from 0.25 wt % to 5.0 wt % will be satisfactory. Greater amounts can be used. A particularly preferred concentration is from 1.0 to 3.0 wt%, especially about 2 wt%.

A PMD-rich extract may be obtained from PMD-containing material, such as the leaves of a eucalyptus plant. A preferred source of PMD rich extract is obtained by stirring eucalyptus citriadora oil derived from the plant with dilute sulphuric acid (usually 5% sulphuric acid), as previously explained in our GB-A-2282534.

In order that the invention may be fully understood, the following examples are given by way of illustration only.

The invention will be described with reference to the accompanying drawings.

Figure 1: Illustrating the isomers of PMD

Figure 2: Illustrating the log reduction in viral titre of HSV-1 virus after treatment with different concentrations of PMD for different contact times

Figure 3: Illustrating the log reduction in viral titre of Urbani SARS virus after treatment with different concentrations of PMD for different contact times

Figure 4: Illustrating the log reduction in viral titre of A/Sydney/5/97 virus after treatment with different concentrations of PMD for different contact times

Two trials were carried out: the first on the influenza virus A/Syney/5/97 (Trial 1); the second on three viruses, namely A/Sydney/5/97, Urbani SARS and HSV-1 (Trial 2).

TRIAL 1

Procedure 1

Procedure for the acute toxicity assay of PMD

The toxicity of PMD at the following concentrations in cell maintenance media was determined on a cell line with MDCK cells:

- 2.5mg/ml (0.25% w/v)
- 5mg/ml (0.5% w/v)
- 20mg/ml (2% w/v)

A cell only control was also implemented by following the same procedure (steps 2-6), but substituting PMD with cell maintenance media.

Toxicity was determined by toxicity-induced CPE (cytopathic effect) observations, which was visually scored using microscopy techniques. Toxicity-induced CPE is characterised by burst or rounded cells, which have become dissociated from their neighbouring cells, or the presence of cellular debris. Toxicity-induced CPE was scored as positive (toxicity observed) or negative (no toxicity observed).

(1) 200µl of each PMD dilution was added to 200µl of cells (at 2 x 10^6 cells/ml) and the reaction incubated for 5 minutes at room temperature.

(2) The reaction was terminated by adding 3.6ml of cell maintenance media appropriate to the cell line.

Note: termination of the reaction is due to the addition of cell maintenance media, which dilutes the reaction 10-fold.

(3) 100µl of the terminated reaction was added to the relevant wells on 48-well plates and incubated for 24 hours at 37°C, 5% CO_2.

Note: the remaining terminated reaction was measured for levels of pH.

(4) The cells in the 48-well plate were trypsinised and a viable cell count was performed using the Trypan blue dye. The percentage of viable cells was used to determine the toxic concentration of PMD in comparison to the cells only control.

Procedure 2

Procedure for the virucidal assay of PMD

The virucidal assay was carried out using four different concentrations of PMD, which do not exhibit toxicity (based on the data obtained from the procedure described in 1).

The four concentrations are as follows.

- 2% w/v (20mg/ml)
- 0.5% w/v (5mg/ml
- 0.25% w/v (2.5mg/ml)
- 0.1% w/v (1mg/ml)

The stock virus was used at a titre greater than 10^4 $TCID_{50}$/ml.

The appropriate positive anti-viral control compound was citric acid.

The presence and absence of viral infection was determined by infection-induced CPE observation, which was visually scored using microscopy techniques. Infection-induced CPE differs between viruses, but is normally characterised by ballooning or rounded cells that remain attached to their neighbouring cells. It was scored as positive (infection apparent) or negative (infection not apparent).

(1) MDCK cell lines were cultivated according to the current Retroscreen Virology Ltd. SOP onto 96-well plates.

(2) 40µl of A/Sydney/5/97 virus was added to 360µl of each PMD dilution and citric acid.

Note: The virus was diluted 10-fold in this step.

(3) The reactions were incubated at room temperature for the following contact times:

- 10 seconds
- 30 seconds
- 1 minutes
- 5 minutes

(4) At each contact time point, the reaction was terminated by adding 3.6ml of infection media appropriate to the cell line.

Note: termination of the reaction is due to the addition of infection media, which dilutes the reaction 10-fold.

(5) 100µl of the terminated reaction was added to the first column of 96-well plates (prepared in point 1) and titrated across the plate following a 1/10 dilution series.

Note: the remaining terminated reaction was measured for levels of pH.

(6) The cells were incubated for 3-5 days at 37°C, 5% CO_2.

(7) CPE was scored daily on the plates to determine the presence or absence of infection. The reduction in viral titre (as a result of anti-viral activity of PMD and the positive control compound, citric acid) was determined.

The results showed that using an influenza virus A/Sydney/5/97 on MDCK cells, there was no viral replication as evidenced by cell survival with 2% w/v PMD in the culture medium. At lower concentrations (0.5%, 0.25% and 0.1%) cells were killed indicating no virucidal effect at these levels.

TRIAL 2

Procedure 1

Procedure for the acute toxicity assay of PMD

The viruses used in the study were:

- HSV-1 (Herpes Simplex Virus type 1)
- Urbani SARS
- A/Sydney/5/97 (human influenza virus H3N2)

PMD was tested at the following concentrations:

- 2% w/v (20mg/ml)
- 1% w/v (10mg/ml)
- 0.5% w/v (5mg/ml
- 0.25% w/v (2.5mg/ml)

The cell line appropriate for each virus is shown in Table 1.

Table 1: Viruses and their appropriate cell lines	
Virus	**Cell Line**
HSV-1	Vero
Urbani SARS	C1008
A/Sydney/5/97	MDCK

Each PMD concentration was made up in 100% isopropyl and then sufficient cell infection media added such that the final concentration of isopropyl was always 10%.

The different concentrations were made up by taking into account the initial dilution of the compound that occurs in the toxicity assay (step 1) and virucidal assay (step 4). The dilution factors for both assays are 2 and 1.1, respectively. Table 2 details the initial PMD concentrations made up for both assays.

Table 2: initial PMD concentrations for the toxicity and virucidal assays and the dilution factor the compound undergoes for each assay		
Final PMD concentration (% w/v)	**Initial PMD concentration (% w/v)**	
	Toxicity assay	**Virucidal assay**
0.25	0.5	0.28
0.5	1	0.56
1	2	1.11
2	4	2.22

Each of the four PMD concentrations was tested for toxicity on each of the three cell lines.

A "cell only" control was also implemented by following the same procedure but substituting PMD (step 1) with infection media.

The procedure for the toxicity assay was as follows:

1) Cells (200µl), at 2 x 10^6 cells/ml, were added to PMD (200µl) and the reaction incubated for 5 minutes at room temperature.
2) The reaction was terminated by the addition of infection media (3.6ml) appropriate to the cell line.

3) The terminated reaction (1ml) was added, in triplicate, to the relevant wells of a 24-well plate.

4) The cells were incubated for 24 hours at 37°C, 5% CO_2.

5) After the incubation period, the cells were trypsinised, the appropriate triplicate wells pooled together and a viable cell count performed using Trypan blue dye.

6) The toxicity of PMD was determined by calculating the percentage cell survival of the test cells in comparison to the control cells.

The results of the toxicity assay for the different concentrations of PMD are shown in Table 3.

Table 3: Percentage cell survival of three different cell lines treated with either 10% isopropyl or three different concentrations of PMD

Cell Line	* Percentage cell survival (%)			
	10% Isopropyl	PMD concentrations (% w/v)		
		0.25	0.5	2
C1008	100	80	60	100
MDCK	40	40	100	85
Vero	100	65	65	100

* values are rounded to the nearest 5

The toxicity of 10% isopropyl was tested to eliminate it as a possible toxic component of the final preparation of PMD.

The 1% w/v PMD concentration was not tested in this assay.

Procedure 2

Procedure for the virucidal assay of PMD

Two types of controls were used in the virucidal assay:

- A positive anti-viral control compound (detailed in Table 4)
- A diluent control – 10% isopropyl – was used as the solvent in the preparation of each PMD concentration. Therefore, it had to be made certain

that this reagent did not possess any virucidal activity against any of the viruses.

Table 4: Viruses and their appropriate positive control compounds for the virucidal assay

Virus	Positive control compound
HSV-1	100% DMSO
Urbani SARS	1% Triton-X
A/Sydney/5/97	Citrate buffer, pH 3.5

The non-toxic PMD concentrations were tested for their virucidal activity against each of the three viruses.

Each virus stock was used at a titre of at least 10^3 $TCID_{50}$/ml.

The procedure for virucidal assay was as follows:

Preparation of 96-well plates

1) Each cell line (3 x 10^5 cells/ml) was seeded onto 96-well plates and left to incubate for 24 hours or until they were 80% confluent.
2) The maintenance media on the plates was removed and the cell monolayers washed with PBS.
3) Infection media (100µl) appropriate to each cell line was added to the plates.

Preparation of the virucidal reaction

4) Virus (40µl) was added to each PMD concentration (360µl) as detailed in procedure 1.
5) The test reactions were incubated at room temperature for the following contact times:

- 10 seconds

- 30 seconds
- 60 seconds (1 minute)
- 300 (5 minutes)

6) After each contact time, the reactions were terminated by the addition of infection media (3.6ml) appropriate to the cell line.

Titration and incubation

7) The infection media in the first column of wells of the 96-well plates (prepared in step 1-3) was removed and replaced with the terminated reactions (110µl), which were plated in duplicate.

8) The terminated reactions were then titrated across the plate following a 10-fold dilution series.

9) The cells were incubated for 5 days at 37°C, 5% CO_2.

10) CPE was scored daily from day 3 post-infection, until day 5 post-infection. In addition, a haemagglutination assay was carried out on day 5 post-infection for the A/Sydney/5/97 virucidal assay only.

11) Any reduction in viral titre for each PMD concentration at each time point, and for the control compounds, was calculated by comparison with the "virus only" control.

12) The assay was also carried out for the antiviral control substances and 10% isopropyl against each virus for the 5 minute contact time.

Tables 5, 6 and 7 show the results of the virucidal assays for HSV-1, Urbani SARS virus and A/Sydney/5/97, respectively.

The results indicate the log reductions in viral titre of each virus in the presence of different PMD concentrations, for different contact times.

A reduction of 1 $\log_{10}$ $TCID_{50}$/ml or greater, (Oxford, J.S. *et al*, 1994) is considered significant for this assay, and is equivalent to a 90% reduction in viral titre.

Table 5: log reductions in viral titre of HSV-1 after treatment with PMD at different concentrations for different contact times

PMD concentration (% w/v)	Log reduction in viral titre (-$\log_{10}$ $TCID_{50}$/ml) PMD contact time (seconds)			
	10	30	‡60	†300
0.25	0	0	0	0.5
0.5	0	0	0	0
1	0	0.5	0.5	1.5
2	0	0	0.5	2.5

‡ 1 minute

† 5 minutes

Table 6: log reductions in viral titre of Urbani SARS after treatment with PMD at different concentrations for different contact times

PMD concentration (% w/v)	Log reduction in viral titre (-$\log_{10}$ $TCID_{50}$/ml) PMD contact time (seconds)			
	10	30	‡60	†300
0.25	1	2	1.5	2
0.5	1.5	2.5	2.5	2.5
1	2	1.5	2	1.5
2	1.5	1	1.5	1.5

‡ 1 minute

† 5 minutes

Table 7: log reductions in viral titre of A/Sydney/5/97 after treatment with PMD at different concentrations for different contact times

PMD concentration (% w/v)	Log reduction in viral titre (-$\log_{10}$ $TCID_{50}$/ml) PMD contact time (seconds)			
	10	30	‡60	†300
0.25	0.4	1.9	1.9	1.4
0.5	0.4	1.9	2.4	1.9
1	1.9	2.4	2.4	2.4
2	1.9	2.4	2.4	2.4

‡ 1 minute

† 5 minutes

The kill rates of different concentrations of PMD are illustrated in Table 8.

The kill rate of PMD against HSV-1 at the 0.25% w/v and 0.5% w/v concentrations is not shown as the results for virucidal assay of this virus indicate that the compound did not exhibit any antiviral activity at these concentrations.

Table 8: Kill rate of different concentrations of PMD against Urbani SARS virus, A/Sydney/5/97 and HSV-1

PMD concentration (% w/v)	Kill rate (-$\log_{10}$ $TCID_{50}$/ml/min)		
	HSV-1	Urbani SARS	A/Sydney/5/97
0.25	-	4.0	4.5
0.5	-	6.0	4.8
1	0.3	6.0	12.0
2	0.5	6.0	12.0

The kill rate values were calculated from the gradients of the lines plotted in Figure 2, Figure 3 and Figure 4, which graphically represent the results obtained for the HSV-1, Urbani SARS virus and A/Sydney/5/97 virucidal assays, respectively. The figures illustrate the log reductions in viral titre of the viruses, in the presence of different concentrations of PMD over time.

Figure 3 and Figure 4 do not show data points for the 5 minute contact time because the results at this time point plateau and show no further significant change.

The measurements of the line gradients were taken after or about the 1-$\log_{10}$ $TCID_{50}$/ml point, as the data before this point are deemed non-significant for the virucidal assay.

RESULTS

HSV-1 Virucidal Assay

The results in Table 5 indicate that the virucidal activity of PMD is time and concentration dependent against HSV-1.

Significant reduction in viral titre was observed for the 1% w/v and 2% w/v concentrations at the 5 minute contact time only. For all other concentrations and contact times, no significant reduction in viral titre was observed.

Although the kill rate of PMD against HSV-1 is not as high as that of the Urbani SARS and A/Sydney/5/97 viruses, it still follows the same trend with the kill rate increasing with increasing PMD concentration. As indicated in Table 8, the kill rate of PMD for HSV-1 has almost doubled from the 1% w/v concentration to the 2% w/v concentration.

The measurement of kill rate for HSV-1 was taken from between the 1 minute and 5 minute time-points. Figure 2 illustrates a gradual increase in log reduction between the 1 minute and 5 minute time points.

Urbani SARS Virucidal Assay

Table 6 shows that all four concentrations of PMD exhibit significant reductions in viral titre at all contact times. It also shows that the compound exhibits neither a time-dependent nor a concentration-dependent activity against the virus.

The kill rates of PMD against Urbani SARS virus, as illustrated in Table 8, are high. However, neither increasing the concentration of PMD nor the contact time, increased the effectiveness of PMD as a virucide, as each concentration produced similar kill rates.

A/Sydney/5/97 Virucidal Assay

The results in Table 7 show that PMD significantly reduces A/Sydney/5/97 infection at all concentrations tested, except 0.25% w/v and 0.5% w/v at the 10 second contact time.

The 1% w/v and 2% w/v concentrations reduced the viral titre by 1.9-2.4 $\log_{10}$ $TCID_{50}$/ml at all contact time points, whereas the two lower concentrations achieved this at the 30 second to 5 minute contact times only.

Table 8 gives an indication of kill rate of A/Sydney/5/97 over a minute. The kill rate of PMD at all concentrations is high and almost triples from the 0.5% w/v concentration to the 1% w/v concentration.

It will be appreciated that the invention may be modified within the scope of the appended claims.

CLAIMS

1. The use of p-menthane-3,8-diol (PMD) in the manufacture of a medicament for use as an antiviral agent.

2. The use of PMD in the manufacture of a medicament for the treatment of diseases caused by viruses having a lipid envelope.

3. The use of PMD as an antiviral agent in non-therapeutic, non-surgical and non-diagnostic applications.

4. The use according to claim 1, 2 or 3, wherein the PMD is used to treat influenza.

5. The use according to claim 1, 2 or 3, wherein the PMD is used to treat influenza caused by the virus A/Sydney/5/97.

6. The use according to claim 1, 2 or 3, wherein the PMD is used to treat Urbani Severe Acute Respiratory Syndrome (Urbani SARS).

7. The use according to claim 1, 2 or 3, wherein the PMD is used to treat Herpes caused by Herpes Simplex virus type-1 (HSV-1).

8. The use of PMD to destroy or inactivate viruses.

9. The use according to claim 8, wherein the virus has a lipid envelope.

10. The use according to claim 8, wherein the virus is influenza.

11. The use according to claim 8, wherein the virus is influenza caused by the influenza virus A/Sydney/5/97.

12. The use according to claim 8, wherein the virus is Urbani SARS.

13. The use according to claim 8, wherein the virus is Herpes caused by HSV-1.

14. The use according to any preceding claim, wherein the PMD is a crude or purified natural product or is a synthetic product.

15. The use according to any preceding claim, wherein the PMD is provided in the form of PMD-rich extract derived from lemon eucalyptus.

16. The use according to any preceding claim, wherein the PMD is provided in the form of a spray.

17. The use according to any preceding claim, wherein the PMD is provided in the form of a composition comprising PMD and a carrier.

18. The use according to any preceding claim, wherein the amount of PMD in the composition is at least 0.25% w/v.

19. The use of PMD substantially as herein described with reference to and as shown in the examples.

ABSTRACT

ANTIVIRAL COMPOSITION

The use of p-menthane-3,8-diol (PMD) as an antiviral agent.

Figure 1

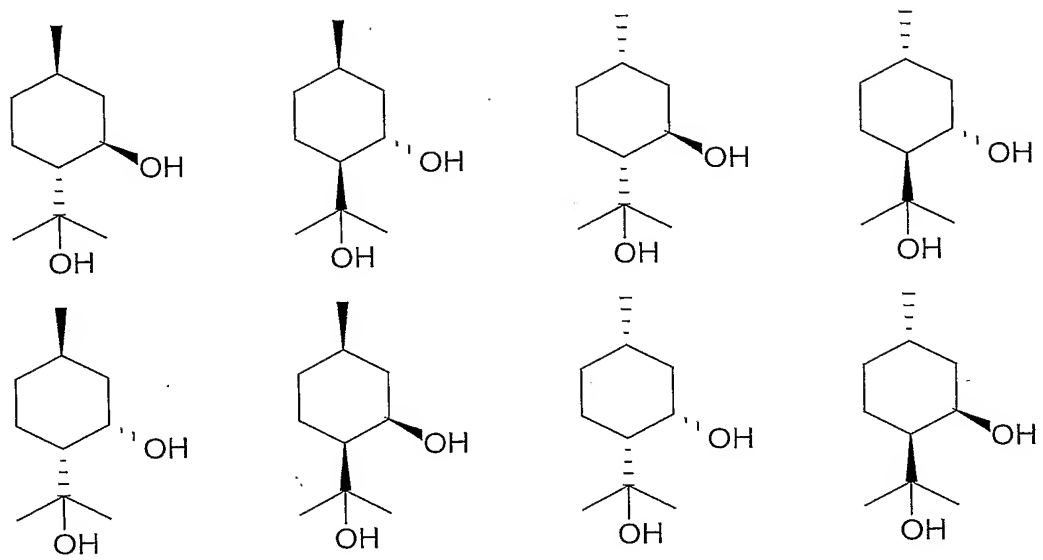

Figure 2

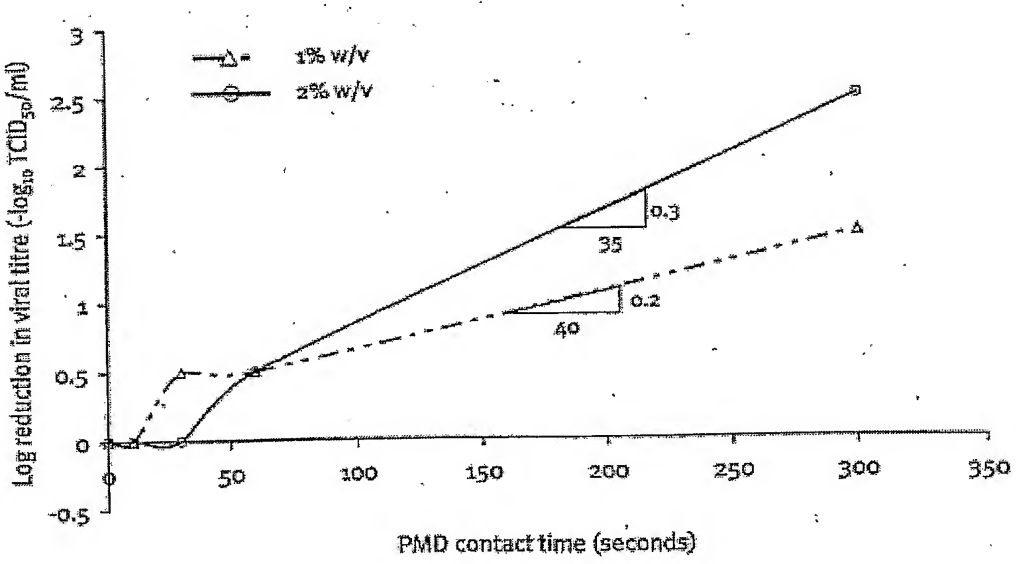

Figure 3

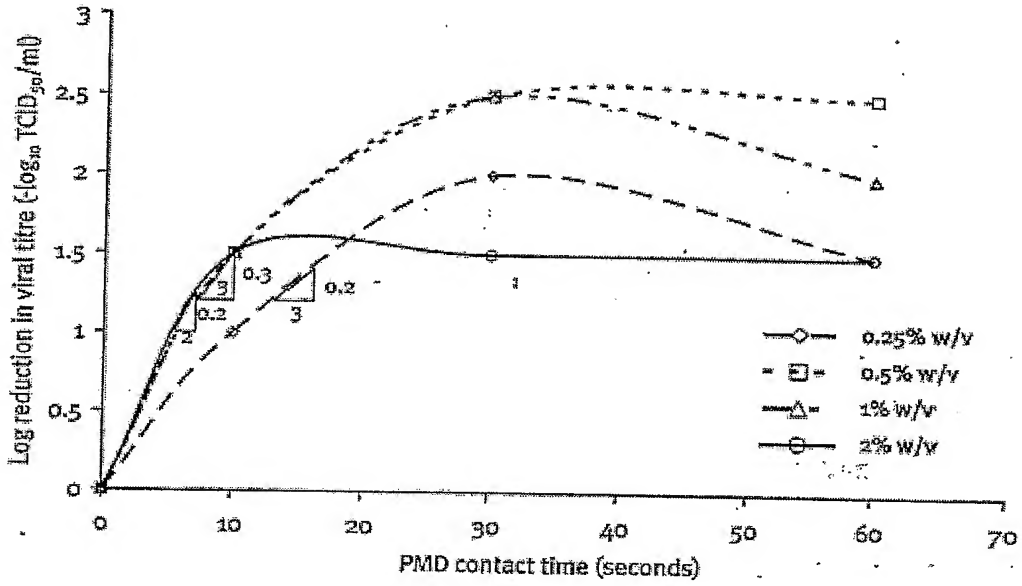

Figure 4